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Dietary threonine requirement of Atlantic salmon smolts

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ABSTRACT

This study was conducted to determine the threonine requirement of Atlantic salmon (*Salmo salar*) smolts. Five diets containing 10.6, 11.7, 13.4, 14.9 and 16.6 g kg^{-1} threonine (presented in protein-bound form of threonine) were fed hourly to satiation to triplicate groups of smolts during the first 7 weeks in seawater. Average initial body weights were 79 ± 2 g per salmon (42 fish per tank). Feed intake was measured daily and growth rates were measured over the trial period. Body composition was analyzed in three groups of 10 fish at the start of the trial and in all of the fish in each group at the end of the trial. Based on the exponential threonine gain response to increasing dietary threonine concentrations, the minimum threonine requirement was found to be 13.1 g kg^{-1} dry matter (15.4 g kg^{-1} free form). The efficiency of digestible threonine utilization for growth in the deficient diets was 0.87.

1. Introduction

When transferred to seawater, Atlantic salmon (*Salmo salar*) smolts are exposed to a new array of environmental and disease conditions. As with many other amino acids, threonine is involved in immune function, as well as for maintaining adequate feed intake, growth and feed efficiency (Bodin et al., 2008; Li et al., 2007; Rodehutscord et al., 1995; Tibaldi and Tulli, 1999). In Atlantic salmon, the threonine requirement has only been determined for fry. Based on nitrogen or threonine gain, the threonine requirement of fry has been reported to be 9.0 to 10.3 g kg^{-1} dry matter (DM) (Bodin et al., 2008; Rollin et al., 2003) (all amino acids will be reported in the protein-bound form unless otherwise indicated). The requirement for rainbow trout (*Oncorhynchus mykiss*) is similar to this (8.8 to 9.0 g kg^{-1}), but that for European sea bass (*Dicentrarchus labrax*) is somewhat higher (12.3 g kg^{-1}) (Bodin et al., 2008; Rodehutscord et al., 1995; Tibaldi and Tulli, 1999). Using a nonlinear N-utilization model, Liebert (2009) reported that the optimal threonine level in diets for Nile tilapia (*Oreochromis niloticus*) may range from 5.0 to 9.9 g kg^{-1} depending on the efficiency of utilization of the amino acid and the protein deposition rate.

The purpose of the present paper was to estimate the dietary requirement of Atlantic salmon smolts for threonine and to examine the effects of threonine deficient diets on the maintenance requirement and the efficiency of utilization of threonine for growth.

2. Materials and methods

2.1. Diets

Five extruded diets (3-mm pellets) were produced by Nofima AS (Fyllingsdalen, Norway). The diets were based on fish meal and wheat gluten and a mixture of crystalline amino acids (Table 1). The diets were supplemented with 0, 2, 4, 6 or 8 g L-threonine kg^{-1} diet. The increase in threonine concentration was compensated by decreases in crystalline asparagine and alanine. The synthetic amino acids were dissolved in a 1% aqueous agar solution (Armisen and Galatas, 1987). The liquid was adjusted to pH 6.8 with 2 M NaOH and then mixed with the other feed ingredients. The mixture was dried overnight and the pH was readjusted to pH 6.8 prior to extrusion. Yttrium oxide was included in the diets as an inert marker for digestibility determinations. The diets were analyzed for dry matter (DM) (105 °C, until constant weight), crude lipid (Soxtec HT6 after hydrolysis with HCl, Tecator, Höganäs, Sweden), nitrogen (crude protein (CP) = nitrogen × 6.25; Kjeltec Auto System, Tecator, Höganäs, Sweden) and ash (550 °C, overnight). Gross energy was measured using an adiabatic bomb calorimeter (Parr 6300, Parr Instrument Company, Moline, IL, USA). Yttrium was analyzed by inductivity-coupled plasma mass-spectroscopy (ICP) at Bioforsk (Ås, Norway). The amino acids in the diets were analyzed using a Biochrom 30 amino acid analyzer (Cambridge, U.K.) following the EC Commission Directive 98/64/EC (1999), after hydrolysis in 6 N HCl for 23 h at 110 °C. Tryptophan and tyrosine were analyzed after basic hydrolysis (Hugli and Moore, 1972). All amino acid concentrations in this paper are presented in their anhydrous, protein-bound forms, unless otherwise indicated.

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Table 1
Formulation and chemical composition of the experimental diets.

Diet	1	2	3	4	5
Ingredients (g kg^{-1})					
Fish meal[a]	235.2	235.2	235.2	235.2	235.2
Wheat gluten[b]	235.2	235.2	235.2	235.2	235.2
Wheat starch	119.6	119.6	119.6	119.6	119.6
Fish oil[c]	236.2	236.2	236.2	236.2	236.2
Amino acid mix[d]	125.4	125.4	125.4	125.4	125.4
L-Threonine[e]	0	2.0	4.0	6.0	8.0
L-Asparagine/L-alanine[f]	8.0	6.0	4.0	2.0	0
Vitamins[g]	20.0	20.0	20.0	20.0	20.0
Minerals[g]	4.0	4.0	4.0	4.0	4.0
Monocalcium phosphate	15.0	15.0	15.0	15.0	15.0
Carophyll®Pink[h]	0.2	0.2	0.2	0.2	0.2
Yttrium oxide	0.1	0.1	0.1	0.1	0.1
Agar	1.1	1.1	1.1	1.1	1.1
Chemical composition (g or MJ kg^{-1})					
Dry matter (DM)	949.5	945.4	950.0	945.1	938.9
In DM					
Crude protein	516.6	514.8	512.9	515.7	521.1
Crude lipid	271.6	252.1	260.1	255.6	250.8
Ash	59.0	59.3	57.8	53.1	55.7
Carbohydrate (by difference)	152.9	173.8	169.2	175.6	172.4
Sum amino acids[i]	430.4	412.4	418.1	420.7	423.1
Threonine[i]	10.6	11.7	13.4	14.9	16.6
Energy	25.2	25.0	24.8	25.1	24.9

[a] Norwegian spring-spawning herring (LT-meal); Karmsund Fiskemel AS, Avaldsnes, Norway.
[b] Amytex 100; Syral, Aalst, Belgium.
[c] NorSalmOil; Norsildmel, Fyllingsdalen, Norway.
[d] Amino acid mixture (% of mixture): L-Lys-HCl 21.9, L-Gln 18.2, L-Arg 8.8, L-Leu 7.4, L-Val 6.6, DL-Met 6.0, L-Pro 5.9, L-Ile 5.0, L-His 4.9, L-Phe 3.4, L-Ser 3.3, L-Gly 3.1, L-Trp 2.1, L-Tyr 2.2, L-Cys 1.1. All amino acids except cysteine (purchased from Sigma) were provided by Ajinomoto Eurolysine S.A.S.
[e] L-Threonine (Ajinomoto Eurolysine S.A.S.).
[f] L-Asparagine and L-alanine each supplied 50% of the mixture w/w (Ajinomoto Eurolysine S.A.S.).
[g] As described by Mundheim et al. (2004).
[h] F. Hoffman-La Roche Ltd., Basel, Switzerland.
[i] Protein-bound forms.

Table 2
Amino acid composition of the experimental diets (% sum protein-bound amino acids).

Dietary Thr (g kg^{-1})	10.6	11.7	13.4	14.9	16.6
Diet	1	2	3	4	5
Indispensable amino acids					
Arginine	6.4	6.4	6.4	6.4	6.4
Histidine	2.9	2.8	2.8	2.8	2.8
Isoleucine	4.3	4.3	4.4	4.3	4.3
Leucine	7.3	7.3	7.3	7.3	7.3
Lysine	7.6	7.4	7.5	7.4	7.4
Methionine	3.2	3.1	3.2	3.2	3.2
Phenylalanine	4.5	4.5	4.5	4.5	4.5
Threonine	2.5	2.8	3.2	3.5	3.9
Tryptophan	1.1	1.2	1.1	1.1	1.1
Valine	5.0	5.0	5.0	4.9	4.9
Dispensable amino acids					
Alanine	4.5	4.3	4.2	3.9	3.8
Aspartate[a]	6.1	5.9	5.7	5.5	5.3
Cysteine	1.4	1.4	1.4	1.4	1.4
Glutamate[a]	24.8	24.7	24.8	24.8	24.7
Glycine	3.8	3.8	3.8	3.8	3.8
Proline	7.7	8.1	7.7	8.0	8.1
Serine	4.1	4.0	4.1	4.1	4.1
Tyrosine	2.9	3.1	3.0	3.1	3.0

[a] Aspartate represents aspartate and asparagine and glutamate represents glutamate and glutamine.

2.2. Tanks and fish

Groups of 64 Atlantic salmon (*S. salar*) juveniles produced at Nofima As, Sunndalsøra, were randomly allocated to 15 500-L tanks supplied with fresh water (constant 24-h light) 8 weeks before the start of the trial. The fish had been subjected to a short daylight regime (12:12 LD) the previous 7 weeks to stimulate the smoltification period. After a two-day fast, the fish were weighed and groups of 42 fish (initial weight 79.3 ± 2.1 g, mean ± S.D., n = 15) were placed back in the tanks. The water supply was switched to seawater the second day of the trial (10.3 ± 1.2 °C; water flow, 12 L min^{-1}; 33.0 ± 0.5 g L^{-1} salinity).

The effluent water of each tank was led into a wire mesh box to enable sieving of waste feed. In order to minimize leaching of the waste feed, the effluent water was directed to two different areas of the wire box using pinch valves on the water pipes, dependent on whether feeding was occurring. Oxygen saturation was measured weekly and was maintained over 85% with oxygen supplementation. Water temperature and salinity were measured daily.

The diets were fed to triplicate groups of salmon. During the 7-week trial, the fish were fed from automatic feeders once per hour. The waste feed was collected daily, weighed and stored frozen. Taking into account the waste feed level and the percentage recovery of DM from each diet (Helland et al., 1996), the approximate intake of fish in each tank was calculated. The feeding level of each tank was adjusted every second day. At the end of the trial the waste feed from each tank was analyzed for DM content and using this value, the daily feed intake was recalculated. Dead fish were removed daily and weighed. This experiment was carried out in accordance with laws and regulations that control experiments and procedures in live animals in Norway, as overseen by the Norwegian Animal Research Authority.

Feces were sieved from the effluent water of the tanks using automatic collectors (Choubert et al., 1982), during weeks 3 to 5. The feces were stored at −20 °C and then freeze-dried and analyzed for crude protein (N × 6.25), crude lipid, energy, amino acids and yttrium, as described above.

2.3. Sampling and weighing

Three groups of 10 fish were sampled from the conditioned fish removed from the tanks at the initiation of the trial. At the end of the trial, the fish were fasted for 2 days and then all fish in each tank were sampled. The fish were anesthetized (tricaine methanesulfonate, MS 222, Argent Chemical Laboratories Inc., Redmont, WA, USA), killed with a blow to the head, weighed and then stored at −20 °C until analysis. The fish were analyzed for DM, crude protein, crude lipid (without HCl hydrolysis), ash, energy and amino acids, as described above.

2.4. Calculations

Thermal growth coefficient (TGC): $1000 \times [(BW_1^{0.33} - BW_0^{0.33})/\sum \text{day} - \text{degrees}]$, where BW_1 and BW_0 are final and initial body weights.

Feed efficiency ratio: (wet fish gain + dead fish weight)/dry feed intake (FI).

Retention of protein, amino acids and energy, %: $100 \times [(BW_1 \times C_1) - (BW_0 \times C_0)] \times (C_{diet} \times FI)^{-1}$, where C_0 and C_1 are initial and final concentrations in whole body, respectively, and C_{diet} is the concentration in the diets. Amino acid calculations were done using the protein-bound form for both the diet and the whole body.

The sum of amino acids (SumAA) is defined as those amino acids that can be used in protein synthesis, thus excluding hydroxylated forms. The weights of the protein-bound forms of the amino acids were used.

Apparent digestibility: $100 - [100 \times (\%\ \text{feed}_i/\%\ \text{feces}_i)(\%\ \text{feces}_n/\%\ \text{feed}_n)]$, where subscript i stands for indicator and n for nutrient (Maynard and Loosli, 1969).

Geometric mean body weight: $(BW_0 \times BW_1)^{1/2}$.